

WHAT IS CLAIMED:

1. A method of detecting single nucleotide polymorphisms comprising:
 - providing a target nucleic acid molecule;
 - 5 providing an oligonucleotide primer complementary to a portion of the target nucleic acid molecule;
 - providing a nucleic acid polymerizing enzyme;
 - providing a plurality of types of nucleotide analogs;
 - blending the target nucleic acid molecule, the oligonucleotide primer,
 - 10 the nucleic acid polymerizing enzyme, and the nucleotide analogs, each type being present in a first amount, to form an extension solution where the oligonucleotide primer is hybridized to the target nucleic acid molecule to form a primed target nucleic acid molecule and the nucleic acid polymerizing enzyme is positioned to add nucleotide analogs to the primed target nucleic acid molecule at an active site;
 - 15 extending the oligonucleotide primer in the extension solution by using the nucleic acid polymerizing enzyme to add a nucleotide analog to the oligonucleotide primer at the active site to form an extended oligonucleotide primer, wherein the nucleotide analog being added is complementary to the nucleotide of the target nucleic acid molecule at the active site;
 - 20 determining the amounts of each type of the nucleotide analogs in the extension solution after said extending, each type being a second amount;
 - comparing the first and second amounts of each type of the nucleotide analog; and
 - identifying the type of nucleotide analog where the first and second
 - 25 amounts differ as the nucleotide added to the oligonucleotide primer at the active site so that the nucleotide at the active site of the target nucleic acid molecule is determined.
2. A method according to claim 1, wherein each type of nucleotide analog is a dideoxynucleotide analog.
- 30 3. A method according to claim 1, wherein said determining is carried out by electrospraying the extension solution.

4. A method according to claim 3, wherein said electrospraying is carried out with an electrospray device comprising:

a substrate having an injection surface and an ejection surface opposing the injection surface, wherein the substrate is an integral monolith

5 comprising:

an entrance orifice on the injection surface;

an exit orifice on the ejection surface;

a channel extending between the entrance orifice and the exit orifice; and

10 a recess extending into the ejection surface and surrounding the exit orifice, thereby defining a nozzle on the ejection surface.

5. A method according to claim 4, wherein the electrospray device further comprises:

a voltage application system comprising:

15 a first electrode attached to said substrate to impart a first potential to said substrate and

a second electrode to impart a second potential, wherein the first and the second electrodes are positioned to define an electric field surrounding the exit orifice.

20 6. A method according to claim 5, wherein the first electrode is electrically insulated from fluid passing through said electrospray device and the second potential is applied to the fluid.

7. A method according to claim 5, wherein the first electrode is in electrical contact with fluid passing through said electrospray device and the second
25 electrode is positioned on the ejection surface.

8. A method according to claim 5, wherein application of potentials to said first and second electrodes causes fluid passing through said electrospray device fluid to discharge from the exit orifice in the form of a spray.

9. A method according to claim 5, wherein application of potentials to said first and second electrodes causes fluid passing through said electrospray device fluid to discharge from the exit orifice in the form of droplets.

5 10. A method according to claim 4, wherein said electrospray device further comprises:

a porous polymeric material associated with said electrospray device at a location suitable to effect liquid chromatographic separation of materials passing through said electrospray device.

10 11. A method according to claim 4, wherein said substrate has a plurality of entrance orifices on the injection surface, a plurality of exit orifices on the ejection surface with each of the plurality of exit orifices corresponding to a respective one of the plurality entrance orifices, and a plurality of channels extending between one of the plurality of exit orifices and the corresponding one of the plurality of entrance orifices.

15 12. A method according to claim 3, wherein said determining comprises detecting the amounts of each type of the nucleotide analogs in the electrospray.

20 13. A method according to claim 12, wherein said detecting is carried out by mass spectrometry, fluorescence, ion conductivity, liquid chromatography, capillary electrophoresis, nuclear magnetic resonance, colorimetric ELISA, immunoradioactivity, radioactivity, or combinations thereof.

14. A method according to claim 3 further comprising:
passing the extension solution through a metal chelating resin prior to said electrospraying.

25 15. A method according to claim 14, wherein the metal chelating resin is a magnesium chelating resin.

16. A method according to claim 14 further comprising:

passing the extension solution through a molecular weight filter prior to said passing the extension solution through a metal chelating agent and

passing the extension solution through a discharge conduit after said passing the extension solution through a metal chelating agent and before said
5 electrospraying.

17. A method according to claim 16, wherein the molecular weight filter, the metal chelating agent, and the discharge conduit are integral.

18. A method according to claim 3 further comprising:
evaporating water from the extension solution, leaving a residue and
10 sonicating the residue.

19. A method according to claim 1, wherein the target nucleic acid molecule is a double stranded DNA molecule.

20. A method according to claim 1 further comprising:
amplifying the target nucleic acid molecule by polymerase chain
15 reaction prior to said blending.

21. An electrospray system comprising:
an electrospray device comprising:
a substrate having an injection surface and an ejection surface
opposing the injection surface, wherein the substrate is an integral monolith
20 comprising:

an entrance orifice on the injection surface;
an exit orifice on the ejection surface;
a channel extending between the entrance orifice and the exit
orifice; and
25 a recess extending into the ejection surface and surrounding the
exit orifice, thereby defining a nozzle on the ejection surface and
a sample preparation device positioned to transfer fluids to said
electrospray device and comprising:
a liquid passage and

a metal chelating resin positioned to treat fluids passing through the liquid passage.

22. An electrospray system according to claim 21, further comprising:

5 a voltage application system comprising:

a first electrode attached to said substrate to impart a first potential to the substrate and

a second electrode to impart a second potential, wherein the first and the second electrodes are positioned to define an electric field surrounding
10 the exit orifice.

23. An electrospray system according to claim 22, wherein the first electrode is electrically insulated from fluid passing through said electrospray device and the second potential is applied to the fluid.

24. An electrospray system according to claim 22, wherein the first
15 electrode is in electrical contact with fluid passing through said electrospray device and the second electrode is positioned on the ejection surface.

25. An electrospray system according to claim 22, wherein application of potentials to said first and second electrodes causes fluid passing through said electrospray device to discharge from the exit orifice in the form of a
20 spray.

26. An electrospray system according to claim 22, wherein application of potentials to said first and second electrodes causes fluid passing through said electrospray device to discharge from the exit orifice in the form of droplets.

25 27. An electrospray system according to claim 21, wherein the electrospray device further comprises:

a porous polymeric material associated with said electrospray device at a location suitable to effect liquid chromatographic separation of materials passing through said electrospray device.

28. An electrospray system according to claim 21, wherein said substrate has a plurality of entrance orifices on the injection surface, a plurality of exit orifices on the ejection surface with each of the plurality of exit orifices corresponding to a respective one of the plurality entrance orifices, and a plurality of channels extending between one of the plurality of exit orifices and the corresponding one of the plurality of entrance orifices.

29. An electrospray system according to claim 21, wherein the electrospray device further comprises:
a well positioned upstream of and in fluid communication with the entrance orifice.

30. An electrospray system according to claim 29, wherein the liquid passage of the sample preparation device comprises a discharge conduit from which fluid in the sample preparation device is discharged into the well.

31. A system according to claim 21 further comprising:
a molecular weight filter integral with the metal chelating resin.

32. A system for processing droplets/sprays of fluid comprising:
an electrospray system according to claim 21 and
a device to receive fluid droplets/sprays of fluid from the exit orifice of said electrospray device.

33. A system according to claim 32, wherein said substrate has a plurality of entrance orifices on the injection surface, a plurality of exit orifices on the ejection surface with each of the plurality of exit orifices corresponding to a respective one of the plurality of entrance orifices, and a plurality of channels extending between one of the plurality of exit orifices and the corresponding one of the plurality of entrance orifices, said device to receive fluid droplets/sprays comprising:

a daughter plate have a plurality of fluid receiving wells each positioned to receive fluid ejected from a respective one of the exit orifices.

34. A system according to claim 32, wherein said device to receive fluid detects fluorescence, ion conductivity, liquid chromatography, capillary electrophoresis, mass spectrometry, nuclear magnetic resonance, colorimetric ELISA, immunoradioactivity, radioactivity, or combinations thereof.

5 35. An electrospray system comprising:
an electrospray device comprising:
a substrate having an injection surface and an ejection surface
opposing the injection surface, wherein the substrate is an integral monolith
comprising:
10 an entrance orifice on the injection surface;
an exit orifice on the ejection surface;
a channel extending between the entrance orifice and the exit
orifice; and
a recess extending into the ejection surface and surrounding the
15 exit orifice, thereby defining a nozzle on the ejection surface and
a sample preparation device positioned to transfer fluids to said
electrospray device and comprising:
a liquid passage and
a molecular weight filter positioned to treat fluids passing
20 through the liquid passage.

36. An electrospray system according to claim 35, further
comprising:
a voltage application system comprising:
a first electrode attached to said substrate to impart a first
25 potential to the substrate and
a second electrode to impart a second potential, wherein the
first and the second electrodes are positioned to define an electric field surrounding
the exit orifice.

37. An electrospray system according to claim 36, wherein the first electrode is electrically insulated from fluid passing through said electrospray device and the second potential is applied to the fluid.

5 38. An electrospray system according to claim 36, wherein the first electrode is in electrical contact with fluid passing through said electrospray device and the second electrode is positioned on the ejection surface.

10 39. An electrospray system according to claim 36, wherein application of potentials to said first and second electrodes causes fluid passing through said electrospray device to discharge from the exit orifice in the form of a spray.

40. An electrospray system according to claim 36, wherein application of potentials to said first and second electrodes causes fluid passing through said electrospray device to discharge from the exit orifice in the form of droplets.

15 41. An electrospray system according to claim 35, wherein the electrospray device further comprises:

a porous polymeric material associated with said electrospray device at a location suitable to effect liquid chromatographic separation of materials passing through said electrospray device.

20 42. An electrospray system according to claim 35, wherein said substrate has a plurality of entrance orifices on the injection surface, a plurality of exit orifices on the ejection surface with each of the plurality of exit orifices corresponding to a respective one of the plurality entrance orifices, and a plurality of channels extending between one of the plurality of exit orifices and the corresponding
25 one of the plurality of entrance orifices.

43. An electrospray system according to claim 35, wherein the electrospray device further comprises:

a well positioned upstream of and in fluid communication with the entrance orifice.

44. An electrospray system according to claim 43, wherein the liquid passage of the sample preparation device comprises a discharge conduit from which fluid in the sample preparation device is discharged into the well.

5 45. A system for processing droplets/sprays of fluid comprising:
an electrospray system according to claim 35 and
a device to receive fluid droplets/sprays of fluid from the exit orifice of
said electrospray device.

10 46. A system according to claim 45, wherein said substrate has a
plurality of entrance orifices on the injection surface, a plurality of exit orifices on the
ejection surface with each of the plurality of exit orifices corresponding to a
respective one of the plurality of entrance orifices, and a plurality of channels
extending between one of the plurality of exit orifices and the corresponding one of
the plurality of entrance orifices, said device to receive fluid droplets/sprays
comprising:
15 a daughter plate have a plurality of fluid receiving wells each
positioned to receive fluid ejected from a respective one of the exit orifices.

47. A system according to claim 46, wherein said device to receive
fluid detects fluorescence, ion conductivity, liquid chromatography, capillary
electrophoresis, mass spectrometry, or combinations thereof.

20 48. A reagent composition comprising:
an aqueous carrier;
an oligonucleotide primer;
a mixture of nucleotide analogs of different types;
magnesium acetate;
25 a buffer, and
a nucleic acid polymerizing enzyme.

49. A reagent composition according to claim 48, wherein an
excess of the oligonucleotide primer to nucleotide analogs is present in said
composition.

50. A reagent composition according to claim 48, wherein a limited concentration of nucleotide analogs is present in said composition.

51. A reagent composition according to claim 48, wherein the buffer is selected from the group consisting of an ammonium bicarbonate, ammonium acetate buffer, and mixtures thereof.

52. A reagent composition according to claim 48, wherein the nucleic acid polymerizing enzyme lacks 3'-5' exo-nuclease activity.

53. A reagent composition according to claim 48, wherein the composition comprises:

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1-10 μ M of the oligonucleotide primer;

0.1 - 10 μ M of each of the nucleotide analogs of different type;

0.5-4 mM of the magnesium acetate;

10-50 mM of the buffer; and

0.1-5 units of the nucleic acid polymerizing enzyme.